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Histidine decarboxylase gene expression in rat fundus is regulated by gastrin

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The conversion of histidine to histamine by histidine decarboxylase (HDC) is of central importance in the control of vertebrate acid secretion. We have used PCR-generated probes to study the regulation of HDC gene expression in rat fundic mucosa. When circulating gastrin levels were lowered by fasting or elevated by treatment with omeprazole, there were parallel changes in HDC mRNA abundance. However, when animals with elevated gastrin levels were concurrently treated with the gastrin/CCK-B receptor antagonist PD 134308, HDC mRNA levels were not increased. These data are consistent with the hypothesis that HDC gene expression is regulated by gastrin, over the physiological range of circulating hormone concentrations.

Histidine decarboxylase; Gastrin; PCR; mRNA; Gene expression; Rat fundus

1. INTRODUCTION

Histidine decarboxylase (HDC; EC 4.1.1.22) converts histidine to histamine. The latter plays an important regulatory role in many biological systems, including control of gastric acid secretion, inflammatory reactions and central neurotransmission, but even so the control of HDC gene expression is poorly understood. The mucosa of the acid-secreting part of the stomach offers a convenient preparation for studies of the control of HDC gene expression since it is known that (i) HDC is localized to a relatively abundant endocrine cell population, the ECL cell [1], and (ii) the gastric hormone gastrin stimulates histamine release [2], HDC enzyme activity, and also proliferation of these cells [3]. In the present study we have examined the hypothesis that gastrin acts by controlling HDC mRNA abundance. We have employed the polymerase chain reaction (PCR) to generate a cDNA probe for HDC, and have manipulated endogenous gastrin concentrations by varying the pH of the gastric contents. Together with the results obtained using novel gastrin antagonists our data indicate that gastrin acts to control HDC mRNA abundance.

2. MATERIALS AND METHODS

2.1. *Probe generation*

Complementary DNA probes were simultaneously synthesized and radiolabelled with ^{32}P using PCR. Reactions were performed in 100 μl 1 × Taq polymerase buffer (Promega) and contained, dATP, dGTP and dTTP (200 μM; Pharmacia), dCTP (2 μm; Pharmacia), [^{32}P]dCTP (100 μCi; ≦3000 Ci/mmol; Amersham), oligonucleotide primers (50 pmol each), cDNA template (≦2 μg) and Taq DNA polymerase (2 units; Promega). After initial denaturation, 33 cycles of amplification (55°C for 1 min, 72°C for 2 min, 95°C for 1 min) were performed and the probes purified on Qiagen-tip 5 mini-columns (Diagen, Düsseldorf). PCR primers were synthesized on an Applied Biosystems 391 oligonucleotide synthesizer and corresponded to bases 132–156 (sense) and bases 1470–1446 (antisense) of rat HDC cDNA [4] and to bases 393–416 (sense) and bases 1037–1014 (antisense) from the coding region of the rat cytoplasmic β-actin gene [5]. cDNA templates for amplification by PCR were from brain (β actin) or fundus (HDC). The PCR-generated HDC and β-actin cDNA probes had electrophoretic properties consistent with their predicted size (Fig. 1).

2.2. *Quantification of mRNA*

At the end of the treatment periods animals were killed by decapitation, trunk blood collected for gastrin radioimmunoassay [6] and samples of gastric antrum and fundus removed for total RNA extraction as previously described [7,8]. Samples of total RNA (20 μg) were electrophoresed in 1% agarose gels and transferred to nylon membranes (Hybond N, Amersham). Membranes were first hybridized with a 1238 base pair (bp) PCR-generated cDNA probe to HDC, washed and exposed to Kodak X-AR film according to previously published protocols [7,8]. The probe was stripped with boiling 0.1% SDS and the membranes rehybridized with a 644 bp PCR probe to rat cytoplasmic β-actin, washed, exposed, stripped and finally rehybridized with an end-labelled oligonucleotide probe to the rat 18 S ribosomal subunit to monitor for equal gel loading and transfer efficiency. Northern blots were quantified by video densitometry.

2.3. *Experimental protocol*

Four groups of 12 female rat were used (200–250 g). In one group, 6 rats were fed *ad libitum*, and 6 were fasted for 48 h (water *ad libitum*), in individual wire-bottomed cages. In a second group, 6 rats fed *ad libitum* were treated daily by gavage with 400 μmol/kg omeprazole (Hässle, Gothenburg) in 0.25% methyl cellulose for 48 h and 6 rats received vehicle alone. A third group of rats were fasted for

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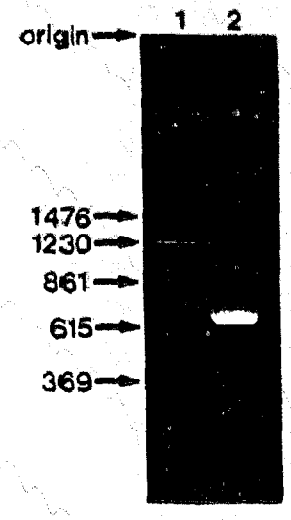

Fig. 1. Electrophoresis of PCR-generated cDNA probes. Reactions were performed as described in the text except that [^{32}P]dCTP was omitted, the products electrophoresed in a 1.5% agarose gel containing ethidium bromide, and visualized with UV light. (Lane 1) HDC cDNA, anticipated size 1238 bp; (lane 2) β-actin cDNA, anticipated size 644 bp. Size markers are from a 123 bp DNA ladder (Gibco BRL, Uxbridge).

48 h, 6 were treated with omeprazole and 6 with vehicle. The fourth group were fasted for 48 h and all 12 received omeprazole treatment; additionally, however, 6 animals received twice daily 4 mg/kg s.c. of the gastrin/CCK-B antagonist PD134308 (Parke-Davies, Cambridge) and 6 received saline subcutaneously.

2.4. *Data analysis*

mRNA abundance is expressed as the ratio of HDC mRNA to that of the non-regulated marker β-actin. Values are given as mean ± SE. Statistical significance was evaluated using a *t*-test.

3. RESULTS AND DISCUSSION

In Northern blots of RNA from rat fundus the HDC probe revealed a major (2.7 kb) and minor (3.5 kb) RNA species (Fig. 2), consistent with previous reports [4]. Only trace amounts of HDC mRNA could be detected in antral samples, presumably reflecting the contribution from mast cells. This distribution is consistent with the localization of HDC mRNA to the ECL population of the fundic mucosa. When animals were fasted for 48 h, fundic HDC mRNA abundance was reduced to 31.2 ± 3.9% (mean ± SE, $n = 6$) of that in control rats fed *ad libitum*. Fasting for 48 h also reduced circulating gastrin concentrations from 53.9 ± 4.7 pmol/l in fed controls to 13.0 ± 3.2 pmol/l ($P < 0.001$). However, when fasted animals were treated with omeprazole at a dose that induces achlorhydria [7], HDC mRNA abundance was significantly elevated 280.7 ± 27.1% above the level in fasted rats that received vehicle ($P < 0.002$, Fig. 2). Omeprazole treatment of fasted rats also elevated plasma gastrin concentrations 8–10-fold compared with vehicle treated fasted animals ($P < 0.01$).

In contrast, omeprazole treatment of fed rats did not significantly alter HDC mRNA abundance (83.3 ± 11.3% of control) although circulating gastrin levels were 2–3-fold higher in omeprazole-treated fed rats compared with vehicle-treated fed controls ($P < 0.01$). We and others have previously shown that when rats are made achlorhydric by treatment with omeprazole or by fundectomy, there is a consequent increase in circulating gastrin concentrations, antral tissue gastrin stores, and gastrin gene expression [7–10]. Omeprazole treatment also increases mucosal histamine stores and histidine decarboxylase activity; these effects are secondary to increased gastrin levels, since they are reversed by antrectomy [3]. The present results indicate a marked increase in HDC mRNA abundance secondary to achlorhydria and so establish control of HDC production by gastric luminal contents. Taken together with the fact that at physiological concentrations gastrin is a potent stimulant of histamine release and

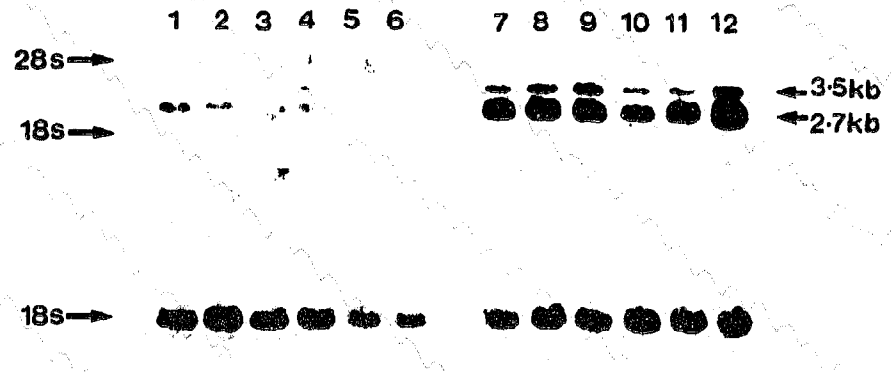

Fig. 2. Northern blot analysis of HDC mRNA and 18 S ribosomal RNA in 20 μg samples of total fundic RNA isolated from control fasting rats (lanes 1–6) or from fasting rats treated with omeprazole (lanes 7–12). The membrane was hybridized first with an HDC cDNA probe (upper panel), secondly with a β-actin cDNA probe, and finally with an 18 S oligonucleotide probe (lower panel). Following hybridizations at 42°C, membranes were washed twice in 2 × SSPE/0.1% SDS at room temperature for 20 min and once in 0.1 × SSPE/0.1% SDS at 65° for 20 min. After exposure to X-ray film, signals were removed with boiling 0.1% SDS. Arrows on the left are 28 S and 18 S ribosomal subunit size markers. Arrows on the right are estimated sizes of HDC mRNA species.

HDC activity in the rat stomach [2,3], these results that gastrin might stimulate HDC gene expression. To test this hypothesis HDC mRNA abundance was measured in fasted omeprazole-treated rats receiving PD134308, which is a potent, orally active, water soluble, antagonist of gastrin/CCK-B receptors ([11] and Campbell and Dimaline, unpublished observations). In rats treated for 48 h with PD134308, HDC mRNA abundance was significantly reduced ($P<0.001$) to $27.2 \pm 5.6\%$ (mean ± SE, $n=5$) that in vehicle-treated animals indicating that endogenous gastrin, at least in part, effects the increase in HDC gene expression induced by achlorhydria.

The changes in HDC mRNA abundance reported here occurred throughout the fasting (≤ 10 pmol/l) to fed (50–100 pmol/l) range of circulating gastrin concentrations, but not above these levels. It seems likely therefore that the control of HDC gene expression occurs within the physiological range of circulating gastrin concentrations. The present results therefore identify one of the endogenous factors that acts to control HDC production in gastric mucosa; it seems likely that other factors control HDC synthesis in mast cells and brain, but the ECL cell offers the opportunity for detailed further studies on the molecular mechanisms of HDC synthesis.

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